

# Genetic Diversity in Annual Xerohalophytes of the Family Chenopodiaceae along Soil Moisture and Salinity Gradients

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**Abstract**—Genetic polymorphism has been studied in populations of two annual xerohalophyte species of the family Chenopodiaceae, *Atriplex tatarica* and *Sedobassia sedoides*, growing on soils with different levels of soil moisture and sodium and potassium ion contents. A tendency toward decrease in the percentage of polymorphic loci in *A. tatarica* at higher soil salinity and increase in the observed heterozygosity of *S. sedoides* populations at higher levels of soil potassium has been revealed, with parameters of genetic variation in either species showing no dependence on soil moisture. Soil potassium deficiency is a stress factor for *S. sedoides* at both physiological and population-genetic levels. Mechanisms controlling sodium absorption and maintaining ion homeostasis and also a high level of homozygosity in *S. sedoides* indicate the improvement of stress tolerance in this annual species.

**Keywords** *слова*: *Atriplex tatarica*, *Sedobassia sedoides*, genetic polymorphism, soil moisture, salinization, potassium deficiency, mechanisms of salt tolerance

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The genetic diversity of a population is a factor determining its stability, adaptation capacity, and possibility to survive under variable environmental conditions. There are many examples of the effect of habitat variation on the genetic diversity and genetic structure of populations [1–4]. Under different environmental conditions, local stress factors may “correct” the basic genetic structure, thereby facilitating local adaptation. The degree of this adaptation is largely determined by the interaction between selection and gene flow between populations along ecological gradients [5, 6]. It is known that the amount of available water in the soil and its contents in plant tissues depend on the concentration of sodium and potassium ions in the soil and their uptake by plants. High salt concentrations in the soil disrupt the homeostasis of water potential and distribution of ions both at the cellular level and in the plant as a whole [7].

Xerohalophyte and halophyte species differ in the capacity for and dynamics of salt accumulation in the aboveground plant parts [8, 9]. The results of our previous study on xerohalophytes of the family Chenopodiaceae in their natural habitats show that they can be divided into two groups with different biochemical protective strategies of salt tolerance in these plants. The first group comprises halophytes in which salt accumulation in shoots depends on soil sodium con-

tent (e.g., *Atriplex tatarica*). Their distinctive feature is significant accumulation of proline, especially in actively growing plants (i.e., plants growing under relatively favorable conditions). In plants of the second group (e.g., *Sedobassia sedoides*), salt accumulation in shoots is independent of salt salinity; i.e., they can take up sodium ions even at a low soil salinity and maintain their concentration at a stable level. It has been found that *S. sedoides* is characterized by a relatively high total flavonoid content in shoots, which is positively correlated with the concentration of potassium ions in tissues [7]. Both these annual species grow on moderately saline and dry soils, and the amounts of biomass they produce in optimal and pessimal habitats differ greatly (by a factor of up to ten), which is evidence for a ruderal adaptive strategy. The influence of local ecological stress factors such as water deficit and soil salinization on the diversity and population structure of xerohalophytes has not yet been studied sufficiently.

The purpose of this study was to analyze the effect of soil moisture and soil sodium and potassium contents on population genetic characteristics (intra- and interpopulation polymorphism) of two annual xerohalophytes, *A. tatarica* and *S. sedoides* (Chenopodiaceae) with different strategies of salt accumulation (salt tolerance).

**Table 1.** Coordinates, characteristics of plant communities, and soil water-salt regime in habitats of *Atriplex tatarica* and *Sedobassia sedoides*

Population no.	Coordinates	Community class	Proportion of species in community, %	Soil moisture, %	Soil Na <sup>+</sup> , mmol/g	Soil K <sup>+</sup> , mmol/g
<i>Atriplex tatarica</i>						
1	52°09.897' N 58°32.522' E	Polygono-Artemisietea austriacae	50 (dominant)	4.5 ± 0.5	0.023 ± 0.001	0.0011 ± 0.0001
2	52°09.897' N 58°32.522' E	Polygono-Artemisietea austriacae	40 (dominant)	1.2 ± 0.4	0.035 ± 0.014	0.0011 ± 0.0001
3	51°51.218' N 58°13.646' E	Polygono-Artemisietea austriacae	40 (dominant)	10.3 ± 0.4	0.006 ± 0.001	0.0062 ± 0.0014
<i>Sedobassia sedoides</i>						
4	52°00.891' N 58°26.690' E	Halimiono verruciferae-Puccinellietum tenuissimae	Single plants	7.1 ± 1.4	0.019 ± 0.005	0.0014 ± 0.0002
5	52°00.891' N 58°26.690' E	Festuco-Puccinellietea	<10	11.7 ± 3.0	0.071 ± 0.023	0.0025 ± 0.0003
6	52°01.186' N 58°28.179' E	Festuco-Puccinellietea	30 (dominant)	6.4 ± 0.7	0.134 ± 0.044	0.0047 ± 0.0010
7	52°09.897' N 58°32.522' E	Polygono-Artemisietea austriacae	10	4.5 ± 0.5	0.023 ± 0.001	0.0011 ± 0.0001
8	51°56.848' N 58°23.344' E	Festuco-Puccinellietea	<10	3.0 ± 1.3	0.090 ± 0.077	0.0101 ± 0.0045

## MATERIAL AND METHODS

Characteristics of the two species included in the study are as follows: *Atriplex tatarica* L. (synonyms *A. laciniata* L., *A. sinuata* Hoffm., *A. veneta* Wild.): annual facultative halophyte, xerophyte [10], ruderal, cosmopolitan plant with C<sub>4</sub> NAD-ME photosynthesis [11] cross-pollinated by wind, chromosome number 2n = 18, 36 [12]; *Sedobassia sedoides* (Pall.) Aschers. (synonyms *Bassia sedoides*, *Echinopsilon sedoides*): annual facultative halophyte, xerophyte, forage plant with intermediate C<sub>3</sub>-C<sub>4</sub> photosynthesis [13] cross-pollinated by wind, chromosome number 2n = 18 [14].

The material for analysis was collected from eight *A. tatarica* and *S. sedoides* populations growing in the Southern Urals in areas with different levels of soil potassium, sodium, and moisture (Table 1).

The seeds and aboveground parts of *A. tatarica* plants were collected in three populations from plant communities nos. 1, 2 and 3 in which this species dominated (Table 1). These communities probably represent one of the final stages in the degradation of steppe pastures, since species of the class Polygono-Artemisietea austriacae play a major role in their structure. The proportion of *A. tatarica* in these communities reached 40–50%, the size of its populations exceeded 100 plants.

The seeds and aboveground parts of *S. sedoides* plants were collected in five populations from communities nos. 4–8. This species dominated only in

community no. 6 (proportion 30%, population size >100 plants), which grows near a populated area and is exposed to grazing. Its composition also includes species of the class Festuco-Puccinellietea characteristic of saline soils. The proportion of *S. sedoides* in two other communities of this class (nos. 5 and 8) was less than 10%, and that in community no. 7 dominated by *A. tatarica* (class Polygono-Artemisietea austriacae) was 10%. Populations of *S. sedoides* in these communities consisted of 50–100 plants. The community dominated by *Halimione verrucifera* (no. 4) on moist solonchaks contained only single *S. sedoides* plants (total population size 15 plants).

The levels of sodium and potassium ions (Na<sup>+</sup> and K<sup>+</sup>) in the upper soil horizon (0–20 cm) and aboveground plant parts (leaves and green shoots) were determined in water extracts from 100-mg samples by atomic absorption spectroscopy using a Hitachi 207 atomic absorption spectrophotometer (Japan). Samples for measuring soil moisture were dried.

Seeds for enzyme analysis in each population were collected from 10–50 plants. To extract enzymes, they were soaked in water for 12 h (until swollen), and embryos were homogenized in 80 μL of Tris-HCl buffer with KCl, MgCl<sub>2</sub>, Triton-X100 and PVP. Enzymes were resolved by electrophoresis in 10% starch gel using two buffer systems [15]: (1) electrode buffer: Tris-citric acid, pH 8.0; gel buffer: 10 mL of electrode buffer + 90 mL of H<sub>2</sub>O, pH 8.0; (2) electrode buffer:

**Table 2.** Genetic parameters of *Atriplex tatarica* and *Sedobassia sedoides* populations

Parameter	<i>A. tatarica</i>	<i>S. sedoides</i>
Proportion of polymorphic loci ( $P$ , %)	64.10 ± 11.72	10.00 ± 10.82
Average allele number per locus ( $A$ )	1.85 ± 0.08	1.16 ± 0.16
Observed heterozygosity ( $H_o$ , %)	7.80 ± 2.72	0.30 ± 0.41
Expected heterozygosity ( $H_e$ , %)	24.70 ± 4.11	2.70 ± 3.15
Inbreeding coefficient of an individual relative to subpopulation ( $F_{is}$ )	0.678	0.882
Inbreeding coefficient of an individual relative to total population ( $F_{it}$ )	0.703	0.939
Index of population differentiation ( $F_{st}$ )	0.078	0.488
Gene flow ( $N_m$ )	2.944	0.263

boric acid–NaOH, pH 8.2; gel buffer: Tris–citric acid, pH 8.7. Electrophoresis was run at 90 V, 40–50 mA (buffer system 1) or 210 V, 70–80 mA (buffer system 2) at 5°C for 4–6 hours. Eight enzymes were included in analysis: glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), diaphorase (DIA, EC 1.6.99), glutamate dehydrogenase (GDH, EC 1.4.1.2), superoxide dismutase (SOD, EC 1.15.1.1), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), 6-phosphogluconate dehydrogenase (6PGD, 1.1.1.44), malate dehydrogenase (MDH, EC 1.1.1.37), and malic enzyme (Me, EC 1.1.1.40). Histochemical staining and genetic interpretation of the results were performed as described [16], with certain modifications. Zymograms of *A. tatarica* samples had no bands formed by dimetric proteins characteristic of tetraploids and were therefore interpreted in the same way as for diploids. The level of genetic variation was assessed by calculating the following parameters: the proportion of polymorphic loci ( $P$ ), average allele number per locus ( $A$ ), and average expected ( $H_e$ ) and observed heterozygosity ( $H_o$ ). Analysis of population structure and gene flow was performed using Wright's  $F$ -statistics ( $F_{is}$ ,  $F_{it}$ ,  $F_{st}$ ) and  $N_m$  [17] in POPGENE 1.32 software [18].

## RESULTS

The populations of *A. tatarica* and *S. sedoides* analyzed for genetic polymorphism were growing on soils with different moisture levels and potassium and sodium ion contents (Table 1). Soil moisture in the study area was very low and did not exceed 12%. Soil sodium content varied widely, from 0.01 to 0.13 mmol/g, with potassium content being ten times lower (0.001–0.010 mmol/g).

Genetic polymorphism under these conditions was higher in *A. tatarica* ( $P = 64.1\%$ ,  $H_e = 24.7\%$ ,  $H_o = 7.8\%$ ) (Table 2). However, the observed heterozygosity was almost three times lower than the expected heterozygosity, indicating considerable heterozygote deficiency in populations ( $F_{it} = 0.703$ ). Polymorphism was observed mainly at the intrapopulation level, with

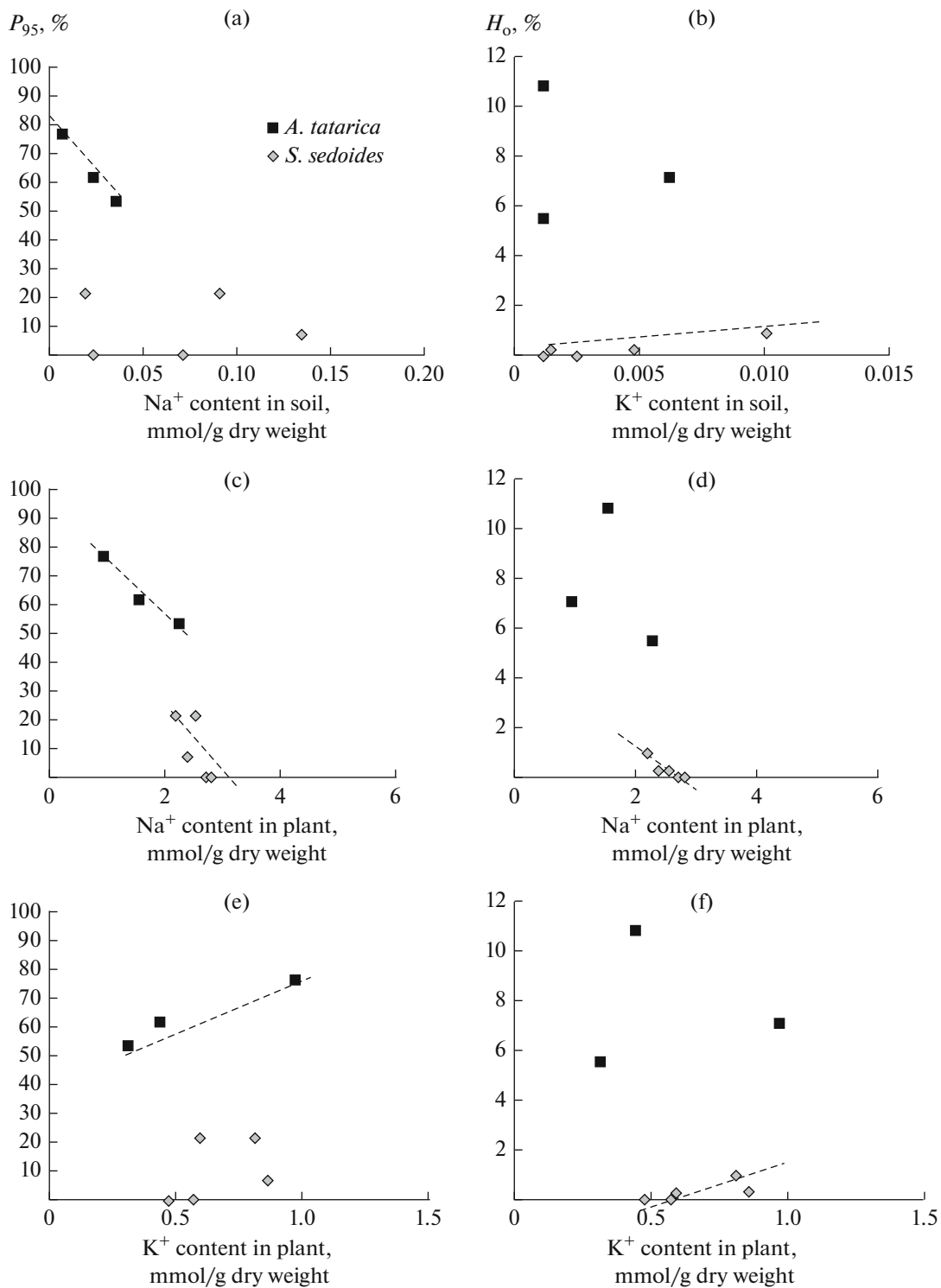
the proportion of variation between populations relative to the total genetic variation ( $F_{st}$ ) being only 7.8%. Populations of *S. sedoides* were characterized by markedly lower genetic polymorphism ( $P = 10\%$ ,  $H_e = 2.7\%$ ,  $H_o = 0.3\%$ ), high heterozygote deficiency (94%), and significant differentiation between them—the proportion of total genetic variation between populations was almost 50% of the total genetic variation—at low gene flow ( $N_m < 1$ ).

Analysis of genetic diversity in the two species along gradients of edaphic factors—soil moisture and sodium and potassium contents—revealed a decrease in the proportion of polymorphic loci with increase in soil salinity in *A. tatarica* (Fig. 1a) and an increase in the observed heterozygosity at higher soil potassium content *S. sedoides* (Fig. 1b). No relationship was observed between parameters of genetic variation and soil moisture level.

Since the accumulation of sodium ions in tissues is the main mechanism of salt tolerance (maintenance of osmotic potential) in *A. tatarica* and *S. sedoides*, we analyzed the level of genetic polymorphism in seeds depending on the contents of  $\text{Na}^+$  (the main osmolyte ions, may be toxic when in excess) and  $\text{K}^+$  (vitaly important ions) in maternal plants. The results showed that, in both species, plants with an elevated tissue sodium content produced seeds with a lower proportion of polymorphic loci (Fig. 1c) and, in *S. sedoides*, lower observed heterozygosity (Fig. 1d). Moreover, an increase in parameters of genetic variation ( $P$  in *A. tatarica* and  $H_o$  in *S. sedoides*) was observed in the seeds of plants accumulating increased amounts of potassium (Figs. 1e, 1f).

## DISCUSSION

Population genetic analysis of two annual xerohalophytes of the family Chenopodiaceae has shown that *A. tatarica* populations are highly polymorphic but weakly differentiated, which is not characteristic of annual species [19]. At the same time, they have simi-



**Fig. 1.** Genetic diversity of two annual xerohalophytes of the family Chenopodiaceae along gradients of (a) sodium and (b) potassium contents in soil and (c, d) sodium and (e, f) potassium contents in plant tissues.

larity with conspecific European populations [20]. Significant gene flow between populations ( $N_m > 1.0$ ) counterbalances genetic drift [17]. Conversely, *S. sedoides* populations growing under similar conditions are weakly polymorphic but strongly differentiated, which

is more typical of annuals. Populations of both species markedly deviate from the Hardy–Weinberg equilibrium toward deficiency in heterozygotes (70–94%). This is uncommon for species cross-pollinated by wind and may be indicative of strong stress pressure

**Table 3.** Edaphic, population genetic, physiological, and ecological characteristics of *Atriplex tatarica* and *Sedobassia sedoides* populations

Conditions and parameters	<i>A. tatarica</i>	<i>S. sedoides</i>
Soil conditions:		
moisture, %	1.2–10.4	3.0–11.7
Na <sup>+</sup> content*	0.01–0.04	0.02–0.13
K <sup>+</sup> content	0.001–0.006	0.001–0.010
Physiological parameters:		
Na <sup>+</sup> in shoots (CV, %)**	0.93–2.27 (43)	2.20–2.82 (10)
K <sup>+</sup> in shoots (CV, %)	0.31–0.97 (61)	0.47–0.86 (25)
Na <sup>+</sup> /K <sup>+</sup> (CV, %)	0.96–7.3 (82)	2.7–6.0 (34)
K <sup>+</sup> + Na <sup>+</sup> (CV, %)	1.9–2.6 (17)	3.01–3.29 (4)
Salt accumulation mechanism***:	Dependent	Independent
ion accumulation range	Wide	Narrow
sum of absorbed ions	Variable	Constant
Na <sup>+</sup> /K <sup>+</sup> ratio	Unstable	Stable
sensitivity	To Na <sup>+</sup> content	To K <sup>+</sup> content
Population genetic parameters:		
polymorphism level	High	Low
population isolation level	Low	High
gene flow	High	Low
Edaphic factor affecting polymorphism	Excess Na <sup>+</sup> affects P <sub>95</sub> level	K <sup>+</sup> deficiency affects heterozygosity
Ecological characteristics:		
adaptive potential	High	Low
stress factor	—	K <sup>+</sup> deficiency
adaptation strategy	Ruderal, cosmopolitan	Ruderal, stress tolerator

\* Na<sup>+</sup> and K<sup>+</sup> contents in soil and plant tissues are given in mmol/g dry weight.

\*\* CV is coefficient of variation.

\*\*\* Dependence of Na<sup>+</sup> accumulation in aboveground plant parts on Na<sup>+</sup> content in soil.

and consequent selection for homozygotes better adapted to extreme conditions [21].

All halophytes depend on a certain level of soil salinity for optimal growth. The study species differ in this respect: *A. tatarica* is tolerant of relatively low sodium concentrations (0.01–0.04 mmol/g soil), whereas the range of salt tolerance in *S. sedoides* is wider (0.02–0.13 mmol/g soil) (Table 3). Halophyte species differ in the capacity for and dynamics of salt accumulation in aboveground plant parts [8, 9]. As shown in our previous study [7], salt accumulation in *A. tatarica* depends on the content of soil sodium. In accordance with this is the observed decrease in the proportion of polymorphic loci under conditions of increasing soil salinity or sodium accumulation in plant tissues (Figs. 1a, 1c).

Although sodium ions play a major role as an osmolyte, their excess may have a toxic effect on the plant as a whole, with consequences for the reproduc-

tive sphere and seed formation [22]. In general, *A. tatarica* is characterized by a wide range of accumulation of sodium and potassium ions (coefficient of variation  $CV = 43–61\%$ ) and unstable Na<sup>+</sup>/K<sup>+</sup> ratio ( $CV = 82\%$ ) (Table 3), which is evidence for low salt tolerance. The decrease in the proportion of polymorphic loci along salinity gradient in this species is probably also related to the functional role of some isozymes in plants affected by the toxic action of sodium. This decrease occurs on account of loci encoding glutamate dehydrogenase (GDH) and glucose-6-phosphate dehydrogenase (G6PD), key enzymes in the regulation of respiratory metabolism under normal conditions and during adaptation to stress [23]. These enzymes reside in regulatory points of the main respiratory pathways: dichotomic glycolysis–tricarboxylic acid cycle (glycolysis–TCA) pathway and apotomic oxidative pentose phosphate pathway (OPPP). The key enzyme of OPPP is G6PD, which catalyzes the

first, oxidative (irreversible) step of this cycle at which the flow of metabolites is redistributed and may be directed along the glycolytic-oxidative pathway [24]. GDH catalyzes  $\alpha$ -ketoglutarate reduction to L-glutamate (in the TCA cycle), a precursor to a number of amino acids, including proline, which plays an important role in plants exposed to drought and salinization [25]. *Atriplex tatarica* accumulates significant amounts of proline, which serves as an antioxidant [7].

*Sedobassia sedoides* has specific mechanisms providing for salt accumulation in plants independently of soil salinity level [7]. This may explain why no correlation of genetic polymorphism with soil salinity was revealed, with its level correlating only with the content of sodium ions in plant tissues (Figs. 1c, 1d). Very low heterozygosity in *S. sedoides* is evidence for prevalence of certain homozygous genotypes that are adapted to specific habitat conditions, particularly in terms of sodium–potassium balance and mechanisms for maintaining ionic homeostasis in tissues. Plants of this species can maintain the  $\text{Na}^+/\text{K}^+$  ratio and total  $\text{Na}^+ + \text{K}^+$  in tissues relatively constant (within a narrow concentration range) independently of soil salinity level (Table 3). The accumulation of potassium ions in plants is limited under these conditions, and potassium deficiency in the soil may hold down their growth and productivity [7]. The role of potassium deficiency as a stress factor for *S. sedoides* is confirmed by reduction in the level of heterozygosity in its populations (Figs. 1b, 1f).

Thus, we have performed analysis of genetic polymorphism in two annual xerohalophyte species of the family Chenopodiaceae with different strategies of salt accumulation (salt tolerance) growing on soils with different levels of soil moisture and sodium and potassium ion contents. The results show that the percentage of polymorphic loci in *A. tatarica* decreases at higher soil salinity and the observed heterozygosity of *S. sedoides* populations increases at higher levels of soil potassium, with parameters of genetic variation in either species showing no dependence on soil moisture. Soil potassium deficiency is a stress factor for *S. sedoides* at both physiological and population-genetic levels. In both species, plants with elevated sodium and decreased potassium contents in tissues produce seeds with a lower percentage of polymorphic loci and, in *S. sedoides*, lower observed heterozygosity. A low level of genetic variation in *S. sedoides* populations may be explained by the possibility (prevalence) of self-pollination, but the absence of correlation between parameters of genetic variation and population density contradicts this assumption.

In our opinion, the high level of heterozygosity and weak differentiation of *A. tatarica* populations suggest a higher adaptive potential of this species, compared to *S. sedoides*, and the absence of correlations with edaphic factors included in analysis indicates that particular habitat conditions are not stressful for *A. tatar-*

*ica*. Population genetic parameters of *S. sedoides* show no correlation with the level of soil salinity, which may be due to the mechanism that maintains the contents of sodium ions in plant tissues at a relatively constant level and thereby effectively protects from their toxic action. On the other hand, potassium deficiency is a stress factor for *S. sedoides* at both physiological and population genetic levels. The mechanisms of sodium absorption control and maintenance of ion homeostasis and the high level of homozygosity in *S. sedoides* populations indicate the improvement of stress tolerance in this annual species.

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#### COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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